Journal of Molecular Diagnostics, Vol. 8, No. 3, July 2006

DOI: 10.2353/jmoldx.2006.050143

Correspondence

Expression of Two Breast-Specific Molecules in the Lung

To the Editor-in-Chief:

We have read with great interest the article by Koga et al citing mammaglobin A as a putative marker for differential diagnosis of lung tumors in patients with a history of breast cancer.[1] *Mammaglobin A* (*MGB1*) was one of the three breast-specific genes identified by these authors following a serial analysis of gene expression (SAGE) database search (*http://cgap.nci.nih.gov/SAGE*). The others two genes were the *small breast epithelial mucin* (*SBEM*) and the *prostate epithelium-specific Ets transcription factor* (*PDEF*). Because the lung is the primary site for breast cancer metastasis, Koga et al tested the efficacy of these candidate genes as potential biomarkers in distinguishing primary lung cancers from metastatic breast cancers.[1] They reported that neither *SBEM* or *PDEF* were useful for differential diagnosis of lung tumors as these two genes are expressed in normal bronchus (21 of 22 samples positive for *SBEM*, 20 of 22 positive for *PDEF*). In contrast, they found all normal lung samples negative for *MGB1*, thereby making it an ideal candidate for identifying breast cancer metastasis to the lung.[1]

It is of particular interest to note that the results of their analysis of the SAGE libraries showed that the ratio of total numbers of breast Tags to lung Tags in normal tissues was higher for MGB1 (9:2) compared to SBEM (376:0).[1] Furthermore, Tags corresponding to MGB1 have been positively identified in a normal lung library whereas Tags corresponding to SBEM have not been detected in this library. In addition, the presence of SBEM mRNA in normal tissues has been investigated by three different laboratories.[2–4] Using Northern dot blot analysis, SBEM mRNA expression was detected in normal breast and salivary glands but not in other normal tissues such as brain, ovary, uterus, prostate, uterus, and lung.[3,4] With a more sensitive microarray hybridization approach, Houghton et al[2] showed that, besides salivary gland and breast, which expressed the highest level of SBEM mRNA, the only other normal tissues expressing detectable levels of this messenger were colon, kidney, and heart but not lung.[2] On the other hand, other laboratories have reported the detection of MGB1 mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR) in primary lung cancers.[5,6]

Altogether, all database analyses and experimental data published have indicated that there is a higher likelihood of detecting MGB1 gene expression rather than SBEM in lung tissues. It was therefore surprising that the data presented by Koga et al showed SBEM to be highly expressed in normal lung tissues (more than 95%) and MGB1 to be undetectable in these samples.[1]

In order to clarify this issue, we analyzed by RT-PCR the synthesis of MGB1 and SBEM mRNAs in matched pairs of normal- and tumor-lung tissue samples collected from 15 patients. These patients with non-small-cell lung cancer (NSCLC) had undergone complete surgical resection of the lung tumor as their primary treatment (ie without prior radiotherapy or chemotherapy) between January 2002 and May 2003 at the 'Hôpital Trousseau' (Tours, France). As shown in Figure 1, no SBEM expression was detectable in any of the 15 normal lung samples studied, while SBEM transcripts were present in three of 15 tumor samples (patients 7, 8, and 11). In addition, MGB1 transcripts were detected in 12 of 15 and 11 of 15 tumor and normal lung tissues, respectively.

These results, in agreement with previous database analyses and experimental data reported by others, do not corroborate the observations of Koga et al. Plausible reasons for such discrepancies might be variations in the cellular composition of the samples, precise pathological evaluation of the tissue samples, contamination of lung samples by skin or technical artifact.

Variations in the Cellular Composition of the Samples

Koga et al observed a difference in *SBEM* gene expression in the different cellular components of the normal lung, with a strong expression in the bronchial surface epithelium and the bronchial gland but no detectable expression in the peripheral parenchyme. Because of sampling irregularities, tissues samples may have included uneven proportion of ciliated epithelial cells, Kulchitsky cells, Clara cells, smooth muscle cells, pneumocyte type I and II, alveolar macrophages, or blood cells. This could affect the outcome of the analysis.

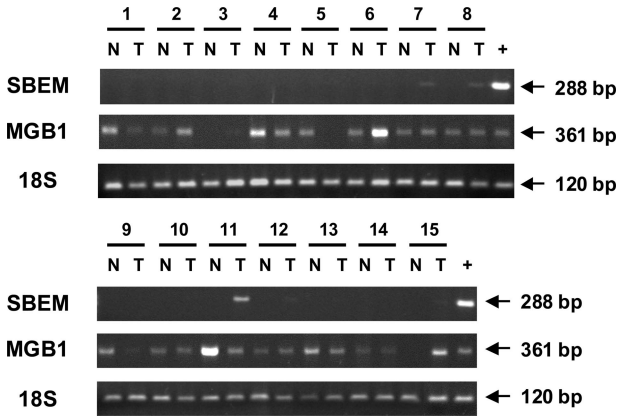

Figure 1. SBEM and MGB1 mRNA expression in normal and tumoral lung tissues. Tumoral and non-tumoral surgical specimens were selected by a pathologist and immediately stored in RNAlater (Ambion, Austin, TX) until RNA extraction and further analysis. Total RNA was isolated, reverse transcribed and PCR amplified as previously described using SBEM-U (5′-gatcttcaggtcaccaccatg-3′) and SBEM-L (5′-gggacacactctaccattcg-3′); Mamma-U (5′-ccgacagcagca-gcctcac-3′) and Mamma-L (5′-tccgtagttggtttctcac-3′); and 18s-U (5′-cgcggttctattttgttggttt-3′) and 18s-L (5′-caagacggaccagagcgaa-3′).[3,7] PCR products were separated on agarose gels and sequenced as described.[3] 18s ribosomal RNA was used as control of cDNA integrity. A pool of four breast tumor cell lines were used as positive control, and for a few randomly selected samples, a negative RT-PCR was performed to confirm the absence of genomic DNA contamination (data not shown). Numbers on top identify patients. N, normal lung; T, tumoral lung; +, positive control.

Pathology of the Tissue Samples

In addition to neoplastic lung tissue samples, normal lung tissue from an area distant to the tumor was identified and dissected by a lung pathologist. The presence of bronchioles and alveoli in normal tissue was further assessed by microscopy of paraffin section (data not shown). These samples may nevertheless differ from normal tissues selected in patients without lung cancer or other diseases. In contrast, Koga et al did not discuss the exact composition of their normal tissues.[1]

Contamination of Lung Samples by Skin Cells

Houghton et al[2] found a detectable expression of *SBEM* by RT-PCR in skin. It is therefore possible that the detection of *SBEM* gene expression in some cases may have resulted from the introduction of skin cells during the process of samples retrieval.

In conclusion, most evidence to date has indicated that MGB1 is more likely to be expressed in normal lung than SBEM and does not support the Koga et al data. Further evaluations need to be carried out to confirm the status of *SBEM* gene expression in lung.

Acknowledgments

We thank Dr. De Muret (Département d'Anatomie Pathologique, CHRU de Tours, France) and Prof. Dumont (Département de Chirurgie Thoracique, CHRU de Tours, France) for providing the lung samples.

This work is supported by the Canadian Institute of Health Research, Friends You Can Count On, the 'Institut pour la Recherche sur la Thrombose et l'Hémostase', and the 'Ligue contre le Cancer'.[2] F.H. is the recipient of a Manitoba Health Research Council award grant; J.R. of a grant from Governmental Research Departement; E.L. of a US Army Medical Research and Materiel Command career development award grant; and Y.M. has a Manitoba Medical Service Foundation career award.

F.H. and J.R. contributed equally to this work.

Florent Hube
Yvonne Myal Etienne Leygue

University of Manitoba
Winnipeg, Manitoba, Canada

Jerome Rollin Yves Gruel

INSERM U618, IFR135
Tours Cedex, France

References

1. Koga T, Horio Y, Mitsudomi T, Takahashi T, Yatabe Y: Identification of MGB1 as a marker in the differential diagnosis of lung tumors in patients with a history of breast cancer by analysis of publicly available SAGE data. J Mol Diagn 2004, 6:90–95
2. Houghton RL, Dillon DC, Molesh DA, Zehentner BK, Xu J, Jiang J, Schmidt C, Frudakis A, Repasky E, Maltez Filho A, Nolasco M, Badaro R, Zhang X, Roche PC, Persing DH, Reed SG: Transcriptional complementarity in breast cancer: application to detection of circulating tumor cells. Mol Diagn 2001, 6:79–91
3. Miksicek RJ, Myal Y, Watson PH, Walker C, Murphy LC, Leygue E: Identification of a novel breast- and salivary gland-specific, mucin-like gene strongly expressed in normal and tumor human mammary epithelium. Cancer Res 2002, 62:2736–2740
4. Colpitts TL, Billing P, Granados E, Hayden M, Hodges S, Roberts L, Russell J, Friedman P, Stroupe S: Identification and immunohistochemical characterization of a mucin-like glycoprotein expressed in early stage breast carcinoma. Tumour Biol 2002, 23:263–278
5. Han JH, Kang Y, Shin HC, Kim HS, Kang YM, Kim YB, Oh SY: Mammaglobin expression in lymph nodes is an important marker of metastatic breast carcinoma. Arch Pathol Lab Med 2003, 127:1330–1334
6. Sjodin A, Guo D, Sorhaug S, Bjermer L, Henriksson R, Hedman H: Dysregulated secretoglobin expression in human lung cancers. Lung Cancer 2003, 41:49–56
7. Guan XF, Hamedani MK, Adeyinka A, Walker C, Kemp A, Murphy LC, Watson PH, Leygue E: Relationship between mammaglobin expression and estrogen receptor status in breast tumors. Endocrine 2003, 21:245–250

Authors' Reply:

We thank Hube et al for their interest in our article. In their analysis of 15 normal and 15 lung cancer tissues, they demonstrated that neither normal lung tissue nor lung cancers expressed mRNA for small breast epithelial mucin (SBEM), whereas the expression of mammaglobin A (MGB1) was frequently detected in those tissues. Those results contradict our findings.

From Northern dot blot hybridization analysis, SBEM was initially reported to be expressed in a restricted range of organs, ie breast and salivary glands.[1] With more sensitive RT-PCR, the range of organs expressing this molecule was extended to include the colon, kidney, and heart.[2] We demonstrated significant expression of SBEM in normal lung tissues,[3] whereas

Table 1. Summary of MGB1 Expression in Normal Lung and Lung Cancers

Authors	Reference no.	Method		MGB1 positive
MGB1 Expression in Normal Lung				
Sjoedin et al	10	NB		Low level
		RT-PCR		0/3 (0%)
Ni et al	11	NB		Negative
Watson et al	5	RT-PCR		Negative
Fleming et al	12	NB		Negative
Zafrakas et al	7	RT-PCR		Negative
Koga et al	3	RT-PCR		0/20 (0%)
Hube et al	This communication	RT-PCR		12/15 (80%)
Yatabe et al	This communication	IHC		0/10 (0%)
MGB1 Expression in Lung Cancers				
Han et al	13	IHC	Lung cancers	5/30 (17%)
Sjoedin et al	10	RT-PCR	NSCLCs	7/15 (46%)
			High-grade NE cancers	4/6 (67%)
Passebosc-Faure et al	14	RT-PCR	Malignant effusion of lung cancers	0/13 (0%)
Bernstein et al	15	IHC	Lung cancers	0/17 (0%)
Zafrakas et al	7	IHC	Lung cancers	1/24 (4%)
Koga et al	3	RT-PCR	NSCLCs	2/60 (3%)
			High-grade NE cancers	5/10 (50%)
Hube et al	This communication	RT-PCR	Lung cancers	11/15 (73%)
Yatabe et al	This communication	IHC	NSCLCs	0/115 (0%)
			High-grade NE cancers	0/3 (0%)

NB, Northern blot hybridization; RT-PCR, reverse transcriptase polymerase reaction; IHC, immunohistochemistry; NE, neuroendocrine; NSCLC, non-small cell lung cancer.

Hube et al reported that SBEM was not expressed in lung tissue. A recent paper by Mitas et al further extended the range of expression to include the esophagus.[4] Unfortunately, we excluded SBEM from further studies because it is not a definitive marker of breast cancer metastasis to the lung. Therefore, we cannot comment on further evidence of SBEM expression in normal lung tissues, in contrast to the expression of MGB1 (see below).

As indicated by Hube et al, it is possible that variation in the cellular composition of tissues may affect the expression status of SBEM. Our analysis with laser microdissection revealed that the main site of SBEM expression is the bronchial surface epithelium and bronchial glands, but not the peripheral parenchyma. However, when lung tissue is obtained, a mixture of bronchus and peripheral parenchyma is common. In our study, the positive expression of SBEM in 21 of 22 normal lungs suggested such a mixed cellular composition. Regarding the second comment, it is quite reasonable that tissue of normal appearance around a tumor may no longer be normal. To obviate this possibility, we included three samples of normal lung tissue that were resected due to metastatic tumors. All these normal lung tissues showed positive expression of SBEM. Regarding the third comment, contamination with skin tissue is unlikely, because the 22 normal lung tissues were obtained immediately after a lobectomy or partial resection at the pathology department, which was separated from the operating theater.

In terms of MGB1 expression, we further confirmed the organ-specific expression of MGB1. Paraffin sections of a total of 473 tumors from various organs were examined with immunohistochemistry. The primary tumors examined included 20 head and neck cancers, eight thyroid cancers, 244 breast cancers, 115 non-small-cell lung cancers, nine esophageal cancers, 19 gastric cancers, 16 colon cancers, three pancreatic cancers, 14 uterine cervical cancers (10 squamous cell carcinomas and four adenocarcinomas), nine endometrial cancers, and 13 ovarian tumors. The expression of MGB1 was restricted to breast and endometrial cancers, whereas none of the 115 lung cancers or 10 normal lung tissue samples were positive for MGB1 (manuscript in preparation). These results are quite consistent with previous work.[5–7] Table 1 summarizes the data on MGB1 expression in normal lungs and lung cancers that have been reported to date, including in this communication. The restricted expression of MGB1 is also supported by other findings reported in the literature. Using expression profiling, Su et al reported a molecular classification based on gene subsets that could individually identify prostate, breast, lung, ovary, colorectal, kidney, liver, pancreatic, bladder, and gastroesophageal cancers.[8] In addition to well-known classifiers such as TTF-1 for lung cancer, MUC-2 for colorectal cancer, and uroplakin II for bladder cancer, MGB1 was selected as a highly specific classifier of breast cancer. Bhattacharjee et al also demonstrated the molecular classification of lung adenocarcinomas with unsupervised hierarchical clustering based on expression profiles. In this analysis, a metastatic cancer from the breast was distinguished by nonlung signatures, including characteristic expression of the estrogen receptor and MGB1.[9] These findings suggest that MGB1 expression in normal lung tissues is quite unlikely, in contrast to the finding of Hube et al. Further evaluation of this issue is required.

Acknowledgment

I thank Yutaka Hatanaka for excellent immunohistochemical staining of MGB1.

Yasushi Yatabe

on behalf of the authors
Aichi Cancer Center
Nagoya, Japan

References

1. Miksicek RJ, Myal Y, Watson PH, Walker C, Murphy LC, Leygue E: Identification of a novel breast- and salivary gland-specific, mucin-like gene strongly expressed in normal and tumor human mammary epithelium. Cancer Res 2002, 62:2736–2740
2. Houghton RL, Dillon DC, Molesh DA, Zehentner BK, Xu J, Jiang J, Schmidt C, Frudakis A, Repasky E, Maltez Filho A, Nolasco M, Badaro R, Zhang X, Roche PC, Persing DH, Reed SG: Transcriptional complementarity in breast cancer: application to detection of circulating tumor cells. Mol Diagn 2001, 6:79–91
3. Koga T, Horio Y, Mitsudomi T, Takahashi T, Yatabe Y: Identification of MGB1 as a marker in the differential diagnosis of lung tumors in patients with a history of breast cancer by analysis of publicly available SAGE data. J Mol Diagn 2004, 6:90–95
4. Mitas M, Almeida JS, Mikhitarian K, Gillanders WE, Lewin DN, Spyropoulos DD, Hoover L, Graham A, Glenn T, King P, Cole DJ, Hawes R, Reed CE, Hoffman BJ: Accurate discrimination of Barrett's esophagus and esophageal adenocarcinoma using a quantitative three-tiered algorithm and multimarker real-time reverse transcription-PCR. Clin Cancer Res 2005, 11:2205–2214
5. Watson MA, Fleming TP: Mammaglobin, a mammary-specific member of the uteroglobin gene family, is overexpressed in human breast cancer. Cancer Res 1996, 56:860–865
6. Grunewald K, Haun M, Fiegl M, Urbanek M, Muller-Holzner E, Massoner A, Riha K, Propst A, Marth C, Gastl G: Mammaglobin expression in gynecologic malignancies and malignant effusions detected by nested reverse transcriptase-polymerase chain reaction. Lab Invest 2002, 82:1147–1153
7. Zafrakas M, Petschke B, Donner A, Fritzsche F, Kristiansen G, Knuechel R, Dahl E: Expression analysis of Mammaglobin A (SCGB2A2) and Lipophilin B (SCGB1D2) in more than 300 human tumors and matching normal tissues reveals their co-expression in gynecologic malignancies. BMC Cancer 2006, 6:88
8. Su AI, Welsh JB, Sapinoso LM, Kern SG, Dimitrov P, Lapp H, Schultz PG, Powell SM, Moskaluk CA, Frierson HF Jr, Hampton GM: Molecular classification of human carcinomas by use of gene expression signatures. Cancer Res 2001, 61:7388–7393
9. Bhattacharjee A, Richards WG, Staunton J, Li C, Monti S, Vasa P, Ladd C, Beheshti J, Bueno R, Gillette M, Loda M, Weber G, Mark EJ, Lander ES, Wong W, Johnson BE, Golub TR, Sugarbaker DJ, Meyerson M: Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci USA 2001, 98:13790–13795
10. Sjodin A, Guo D, Sorhaug S, Bjermer L, Henriksson R, Hedman H: Dysregulated secretoglobin expression in human lung cancers. Lung Cancer 2003, 41:49–56
11. Ni J, Kalff-Suske M, Gentz R, Schageman J, Beato M, Klug J: All human genes of the uteroglobin family are localized on chromosome 11q12.2 and form a dense cluster. Ann N Y Acad Sci 2000, 923:25–42
12. Fleming TP, Watson MA: Mammaglobin, a breast-specific gene, and its utility as a marker for breast cancer. Ann N Y Acad Sci 2000, 923:78–89
13. Han JH, Kang Y, Shin HC, Kim HS, Kang YM, Kim YB, Oh SY: Mammaglobin expression in lymph nodes is an important marker of metastatic breast carcinoma. Arch Pathol Lab Med 2003, 127:1330–1334
14. Passebosc-Faure K, Li G, Lambert C, Cottier M, Gentil-Perret A, Fournel P, Perol M, Genin C: Evaluation of a panel of molecular markers for the diagnosis of malignant serous effusions. Clin Cancer Res 2005, 11:6862–6867
15. Bernstein JL, Godbold JH, Raptis G, Watson MA, Levinson B, Aaronson SA, Fleming TP: Identification of mammaglobin as a novel serum marker for breast cancer. Clin Cancer Res 2005, 11:6528–6535